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TITLE: Studies on the Role of the Ah Receptor (AhR) on the Etiology of Breast Cancer: A Novel Idea of Identifying this Receptor as a New Therapeutic Target

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14. ABSTRACT <p>In this project we have carried out, first, laboratory experiments on cultured human breast cancer cells, particularly an MCF10AT1-derived P20E breast cancer cells which are over-expressing AhR, to ascertain that their expressions of the genetic markers of carcinogenic transformation are essentially identical to those already identified in the AhR over-expressing and ERα under-expressing type of breast tumor samples. Second, we have conducted laboratory experiments in vitro to test the efficacies of several known AhR blocking agents on those AhR over-expressing breast cancer cells, and found that the most potent phytochemical suppressors of cell proliferation of P20E cells were curcumin (10 μM approximately 80 to 90% suppression), zerumbone (10μM, 70 to 90% suppression), and luteolin (5μM, 70 to 85% suppression). The potencies of these naturally occurring chemicals were then compared to a well established blocker of AHR, MNF (10μM) and a suppressor of DNA-methylation, 5-aza-2-deoxycytidine (AZ, at 0.5M). It was found that MNF causes only 50% suppression, while AZ could induce about 70 to 80% suppression. Of those only luteolin and MNF showed consistent inhibitory actions on the function of AhR in vitro. Preliminary studies, on the effects of those chemicals in suppressing the effect of TCDD in live mice, showed that luteolin was the most reliable chemical consistently reducing the action of TCDD in vivo. Finally by using the cultured, AhR over-expressing P20E breast cancer cells, we tested their tumorigenic growth in vivo in athymic nude mice.</p>					
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Final Report on W81XWH-09-1-0553 entitled, Significance of overexpressed arylhydrocarbon receptor on progression of mammary epithelial cells into malignant phenotype

Prepared by Fumio Matsumura, principal investigator, University of California Davis

Introduction

Human breast cancer is a disease that is tremendously affected by the environment. We have discovered recently that ER α -negative and RelB-positive type of breast tumors show high expressions of the Ah receptor (AhR), which has been known for its role to mediate toxicity of dioxin. To study this phenomenon in the laboratory, we have obtained several AhR overexpressing breast cancer cell lines. Treatments of those cells with known AhR antagonists have clearly eliminated their transformation phenotypes such as increased proliferation, anchorage-independent growth, invasiveness into matrigel, epithelial to mesenchymal transition and resistance to apoptosis. Preliminary studies have shown that all of these phenotypic expressions of these cells are significantly reduced by several potent blockers of AhR. Although the existence of this type of inflammatory breast cancers has been known for some time (Van Laere et al. 2006), no one knew the prominent role of AhR plays. Therefore, this finding has provided an opportunity for us to test the possibility of utilizing AhR as the potential therapeutic target.

Hypothesis/Rationale: We hypothesize that AhR is essential in assuring the survival and growth of this type of tumor cells, and that blocking the function of AhR would result in the reduction of the tumor development.

Objectives: (1) To screen a number of AhR blockers to suppress the expression of cancer phenotypes of those AhR overexpressing, human breast cancer cells, and (2) by using the athymic nude mouse model, test the possibility of suppressing the breast tumor growth and metastatic migration of xenografted, AhR overexpressing, human breast cancer cells.

Methods: Methods: Our basic plan has been to test the possibility of using those AhR antagonists as possible therapeutic agents, for the first time. We have done so by testing the effectiveness of a number of phytochemicals from edible plants known to block AhR in attenuating the expression of high rates of cell proliferation and resistance to UV-irradiation induced apoptosis such as: luteolin curcumin, zerumbone and resveratrol. Second we tested the effectiveness of a selected number of those phytochemicals, by xenografting those AhR overexpressing human breast cancer cells into athymic nude mice, and by treating them in vivo with these AhR antagonists, and then after 8 to 10 weeks assessing the extent of reduction of tumor growth.

Results.

In this project we have carried out, first, laboratory experiments on cultured human breast cancer cells, particularly an MCF10AT1-derived P20E breast cancer cells which are over-expressing AhR, to ascertain that their expressions of the genetic markers of carcinogenic transformation are essentially identical to those already identified in the AhR over-expressing and ER α under-expressing type of breast tumor samples. Second, we have conducted laboratory experiments in vitro to test the efficacies of

several known AhR blocking agents on those AhR over-expressing breast cancer cells (Fig.1), and found that the most potent phytochemical suppressors of cell proliferation of P20E cells were curcumin (10 μ M approximately 80 to 90% suppression), zerumbone (10 μ M, 70 to 90% suppression), and luteolin (5 μ M, 70 to 85% suppression). The potencies of these naturally occurring chemicals were then compared to a well established blocker of AHR, MNF (10 μ M). Of those only luteolin and MNF showed consistent inhibitory actions on the function of AhR in vitro. Preliminary studies, on the effects of those chemicals in suppressing the effect of TCDD (=dioxin, the most powerful and persistent activator of AHR used here as the acid test) in live mice (in vivo), showed that luteolin was the most reliable chemical consistently reducing the action of TCDD in vivo (Fig. 2A). Other inhibitors, including CH223191 (a well known blocker of AhR) and zerumbone did not reduce the action of TCDD (dioxin) in vivo (Fig. 2B), despite their effectiveness in vitro on P20E cells. Finally by utilizing the cultured, AhR over-expressing P20E breast cancer cells, we tested their tumorigenic growth in vivo in athymic nude mice. This was done by initially transplanting (i.e. xenografting) those laboratory cultured human breast cancer cells into the fat pad of the breast tissue of live athymic nude mice, and treating them with luteolin, the promising AhR inhibitor (Fig. 3). The effectiveness of luteolin in suppressing tumor growth was studied by conducting continuous physical examinations through out the 10 weeks observation period on luteolin treated, P20E xenografted mice, along with control mice which received only the vehicle used. The differences found between luteolin treated and vehicle-treated (=control) mice in terms of the extents of development of tumors during this observation period were clearly significant.

Key Research Accomplishments

- 1) Identification of zerumbone, luteolin, curcumin, MNF and 5-aza-2-deoxycytidine as 5 most potent suppressors of cell proliferation of P20E human breast cancer cells in vitro.
- 2) Final selection of luteolin to be the most potent blocker of the function of AHR in live mice.
- 3) Establishment of an in vivo model of human breast tumor growth by transplanting (xenografting) P20E human breast cancer cells into athymic nude mice.
- 4) Confirming the effectiveness of luteolin in retarding tumor growth of human breast cancer cells in the above in vivo nude mouse model.

Reportable Outcome

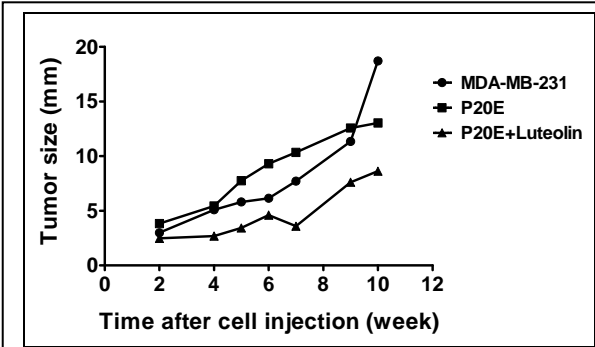
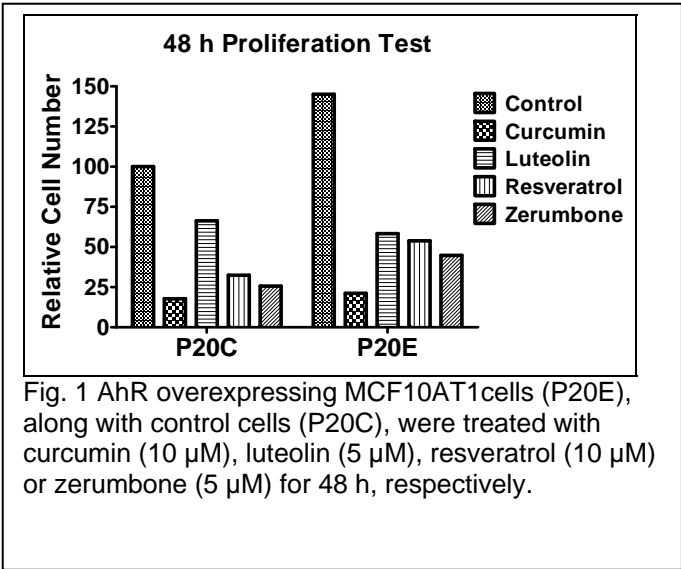
We are in the process of preparing a manuscript based on the above findings. However, we still need additional experiments to confirm these results. As soon as it is completed, reviewed and become a scientifically sound manuscript, which can be submitted to a reputable scientific journal, we will provide that pre-publication material to the sponsor of this project.

Conclusion.

Luteolin, a naturally occurring product from broccoli and other edible plants, has been found to block the function of the arylhydrocarbon receptor (AHR) in live mice.

Furthermore, treatment of athymic nude mice with luteolin clearly retarded the growth of breast tumors that were originated from transplanted AHR overexpressing human breast cancer cells.

Figures



Average tumor size in the nude mice injected with breast cancer cells (MDA-MB-231, AhR overexpressing P20E, and P20E plus luteolin). The difference between P20E versus P20E+luteolin on week 7,9and 10 were significant at $p<0.05$.

